REMARKS

Claims 24-36 have been canceled without prejudice to expedite prosecution of this application. Applicant intends to pursue the broader aspects in a continuation application.

> According to M.P.E.P. §2164.08:
> The Federal Circuit has repeatedly held that "the specification must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation'." In re Wright, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Nevertheless, not everything necessary to practice the invention need be disclosed. <u>In fact, what is well-known is best omitted</u>. In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). All that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art. Further the scope of enablement must only bear a "reasonable correlation" to the scope of the claims. See, e.g., In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970).

(Underlining added)

The sequence of SLIM3 was well-known and readily available to the skilled worker at the time the time the application was filed, and therefore it is unnecessary to incorporate it into the specification. For example, sequences for SLIM2 were disclosed in Morgan and Madgwick, BBRC, 225:632-338; Chan et al., Gene, 210:345-350, 1988 (Exhibit 1; SLIM2 is also know as FHL2 as stated on Page 347); and in various Genbank listings, including U60117 and Q14192 (Exhibit 2). Thus, withdrawal of the requirement to incorporate the sequence into the specification is respectfully requested.

In view of the above remarks, favorable reconsideration is courteously requested. If there are any remaining issues which could be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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EXHIBIT

1

ELSEVIER

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GENE
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Molecular cloning and characterization of FHL2, a novel LIM domain protein preferentially expressed in human heart

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Abstract

A full-length cDNA clone encoding a novel LIM-only protein was isolated and sequenced from a human fetal heart cDNA library. This full-length clone consists of 1416 base pairs and has a predicted open reading frame (ORF) encoding 279 amino acids. The ORF of this polypeptide codes for the human heart-specific four and a half LIM-only protein 2 (FHL2). It possesses an extra zinc finger that is a half LIM domain and four repeats of LIM domain. When the human FHL2 cDNA probe was used to hybridize with poly-A RNA of various human tissues, a very strong signal could be seen in heart tissues, and only moderately low signals could be detected in placenta, skeletal muscle and ovary. Virtually no signal could be detected in brain, lung, liver, kidney, pancreas, spleen, thymus, prostate, testis, small intestine, colon or peripheral blood leukocyte. FHL2 was mapped to chromosome 2q12–q13 by fluorescent in-situ hybridization (FISH). © 1998 Elsevier Science B.V.

Keywords: Heart cDNA; LIM domain protein; Zinc finger protein; Chromosome 2

1. Introduction

Zinc finger proteins can be classified based on different classes of consensus sequences. One class of zinc finger proteins bears a LIM motif that consists of a C_2HC motif and a C_4 motif (Liebhaber et al., 1990). LIM proteins are involved in cell identity, differentiation, and growth control (Dawid et al., 1994; Sanchez-Garcia and Rabbitts, 1994). They can be classified into three subclasses: (1) LIM-homeodomain proteins; (2) LIM-functional domain proteins, for example LIM kinases; and (3) LIM-only proteins. Many muscle-specific LIM proteins have been identified (Arber et al., 1994; Morgan et al., 1995, Morgan and Madgwick, 1996b; Jain et al., 1996), and one of them has been associated with a key role in muscle development (Arber et al., 1994; Arber et al., 1997). In order to study the relationship between the LIM proteins and the differentiation and growth regulation of the heart, we have cloned and characterized three human heart cDNAs that code for LIM-only proteins (Tsui et al., 1994, 1996; Fung et al., 1995, 1996). In this study, we report the cloning and sequencing of a full-length cDNA that codes for a novel heart-specific four and a half LIM-only protein 2 (FHL2). We report here the tissue distribution of FHL2 as revealed by Northern hybridization and the chromosomal mapping of the FHL2 gene by fluorescent in-situ hybridization (FISH). The possible roles of FHL2 are also discussed.

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Abbreviations: aa, amino acid(s); BLAST, Basic Local Alignment Search Tool; FHL2, four and a half LIM-only protein 2; FISH, fluorescent in-situ hybridization; MLP, muscle LIM protein; MRF, muscle regulatory factor; NCBI, National Center for Biotechnology Information; ORF, open reading frame; PCR, polymerase chain reaction; SLIM3, skeletal muscle LIM-protein 3.

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2. Experimental and discussion

2.1. Isolation of the FHL2 cDNA

Partial sequencing of cDNA clones isolated from a directionally cloned human fetal heart (10–12 weeks)

```
   1     C AGA AGG TTG GGG CCA TCC AAC CAG GCA GTC CGC CTG CAC AGC AGC     46

  47   TGT CCC TGC TCA TCG GGC TGG AGG ACA GAA GAC AGA ACC CTA AAA CCA     94

   1                                       M   T   E   R   F   D   C      7
  95   CAG GTT GCT GAA AAG CCA GGA GTC AAA ATG ACT GAG CGC TTT GAC TGC    142

   8    H   H   C   N   E   S   L   F   G   K   K   Y   I   L   R   E     23
 143   CAC CAT TGC AAC GAA TCT CTC TTT GGC AAG AAG TAC ATC CTG CGG GAG    190

  24    E   S   P   Y   C   V   V   C   F   E   T   L   F   A   N   T     39
 191   GAG AGC CCC TAC TGC GTG GTG TGC TTT GAG ACC CTG TTC GCC AAC ACC    238

  40    C   E   E   C   G   K   P   I   G   C   D   C   K   D   L   S     55
 239   TGC GAG GAG TGT GGG AAG CCC ATC GGC TGT GAC TGC AAG GAC TTG TCT    286

  56    Y   K   D   R   H   W   H   E   A   C   F   H   C   S   Q   C     71
 287   TAC AAG GAC CGG CAC TGG CAT GAA GCC TGT TTC CAC TGC TCG CAG TGC    334

  72    R   N   S   L   V   D   K   P   F   A   A   K   E   D   Q   L     87
 335   AGA AAC TCA CTG GTG GAC AAG CCC TTT GCT GCC AAG GAG GAC CAG CTG    382

  88    L   C   T   D   C   Y   S   N   E   Y   S   S   K   C   Q   E    103
 383   CTC TGT ACA GAC TGC TAT TCC AAC GAG TAC TCA TCC AAG TGC CAG GAA    430

 104    C   K   K   T   I   M   P   G   T   R   K   M   E   Y   K   G    119
 431   TGC AAG AAG ACC ATC ATG CCA GGT ACC CGC AAG ATG GAG TAC AAG GGC    478

 120    S   S   W   H   E   T   C   F   I   C   H   R   C   Q   Q   P    135
 479   AGC AGC TGG CAT GAG ACC TGC TTC ATC TGC CAC CGC TGC CAG CAG CCA    526

 136    I   G   T   K   S   F   I   P   K   D   N   Q   N   F   C   V    151
 527   ATT GGA ACC AAG AGT TTC ATC CCC AAA GAC AAT CAG AAT TTC TGT GTG    574

 152    P   C   Y   E   K   Q   H   A   M   Q   C   V   Q   C   K   M    167
 575   CCC TGC TAT GAG AAA CAA CAT GCC ATG CAG TGC GTT CAG TGC AAA ATG    622

 168    P   I   T   T   G   G   V   T   Y   R   E   Q   P   W   H   K    183
 623   CCC ATC ACC ACG GGA GGG GTC ACT TAC CGG GAG CAG CCC TGG CAC AAG    670

 184    E   C   F   V   C   T   A   C   R   K   Q   L   S   G   Q   R    199
 671   GAG TGC TTC GTG TGC ACC GCC TGC AGG AAG CAG CTG TCT GGG CAG CGC    718

 200    F   T   A   R   D   D   F   A   Y   C   L   N   C   F   C   D    215
 719   TTC ACA GCT CGC GAT GAC TTT GCC TAC TGC CTG AAC TGC TTC TGT GAC    766

 216    L   Y   A   K   K   C   A   G   C   T   N   P   I   S   G   L    231
 767   TTG TAT GCC AAG AAG TGT GCT GGG TGC ACC AAC CCC ATC AGC GGA CTT    814

 232    G   G   T   K   Y   I   S   F   E   E   R   Q   W   H   N   D    247
 815   GGT GGC ACA AAA TAC ATC TCC TTT GAG GAA CGG CAG TGG CAT AAC GAC    862

 248    C   F   N   C   K   K   C   S   L   S   L   V   G   R   G   F    263
 863   TGC TTT AAC TGT AAG AAG TGC TCC CTC TCA CTG GTG GGG CGT GGC TTC    910

 264    L   T   E   R   D   D   I   L   C   P   D   C   G   K   D   I    279
 911   CTC ACA GAG AGG GAC GAC ATC CTG TGC CCC GAC TGT GGG AAA GAC ATC    958

 280    *                                                                280
 959   TGA ATT CAA CAC AGA GAA GTT GCT GCT TGT GAT CTC ACA CAC AGA TTT   1006

1007   TTA TGT TTT CTT TCT CAC CCA GGC AAT CTT GCC TTC TGG TTT CTT CCA   1054

1055   GCC ACA TTG AGA CTT TCT TCT AGT GCT TTT CAG TGA TAC TCA CGT TTG   1102

1103   CTT AAA CCC TTT AGT GCT TTG TGA TAG TTC AGT CCC AGG GAA AGA GAA   1150

1151   AAC TCG CCC TAG GCC CTA GGT GGG AAG ATG GTT TGA AAT TTT TGT AAT   1198

1199   CGA GTA AGG CAC ACC CAA ATG TAA AAA TCC TTT TGA ATG ATG CCT TTA   1246

1247   TAA ATC TTT CTC TCA CTG TCT ATT TAA GTG CAA TTA ACA TAT GTC ACG   1294

1295   AAC TTG AAA GTT TTC TAA ACT CAA TAA GGT AAT GAC CAG TTG TTA TTT   1342

1343   ACA GCT CTG TAA CCT CCC GTT GCG TCA AGT CTA AAC CAA GAT TAT GTG   1390

1391   ACT TGC AAT AAA GTT ATT CAG AAC AG                                1416
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Fig. 1. cDNA and predicted amino-acid sequences of FHL2. The nucleotide sequence data has been submitted to the GenBank/EMBL Data Libraries under the accession number U29332. In the DNA sequence, the polyadenylation signal (AATAAA) are underlined. **Methods**: Partial sequencing of cDNAs clones from a directionally cloned human fetal heart (10–12 weeks) library was conducted as described (Hwang et al., 1995; Liew et al., 1994; Tsui et al., 1995). Briefly, eluted phage plaques were subjected to PCR in the presence of primers flanking the restriction sites of the lambda gt22 vector (forward: 5′-ATTGGTGGCGACGACTCCTGGA-3′; reverse: 5′-TTTGACACCAGACCAACTGGTA-3′). PCR products were sequenced directly using a cycle sequencing kit (dsDNA Cycle Sequencing System, Life Technologies) in the presence of 160 nM of a fluorescein-conjugated primer nested within the forward PCR primer (fluorescein-5′-GGTGGCGACGACTCCTGGAGCC-3′). The sequencing products were run and analysed in a Pharmacia A.L.F. DNA sequencer. Sequence comparisons against the GenBank and EMBL nucleotide and protein databases were performed using the BLAST electronic mail server (Altschul et al., 1990). The complete sequence of the cDNA was determined by primer walking strategy using dideoxy sequencing.

cDNA library was conducted as described (Liew et al., 1994; Hwang et al., 1995; Tsui et al., 1995). Briefly, eluted phage plaques were subjected to polymerase chain reaction (PCR) in the presence of primers flanking the restriction sites of the lambda gt22 vector. PCR products were sequenced, and one of the cDNA clones exhibited a DNA sequence similarity to that of the LIM domain protein family. The putative protein encoded by this cDNA is named heart-specific four and a half LIM-only protein 2 (FHL2). The PCR product of FHL2 cDNA clone was subcloned into the plasmid pGBT9. The DNA sequence of the open reading frame of FHL2 was verified by sequencing with T7 DNA polymerase.

Excluding the vector sequence and the poly (A) region, the FHL2 cDNA insert is 1416 base pairs in length. The initiation and stop codons were found at nucleotide numbers 122 and 959, respectively. A typical polyadenylation signal (AATAAA) was found at nucleotide number 1397–1402 (Fig. 1).

2.2. Sequence analysis of FHL2

After translating the open reading frame (ORF) of the FHL2 cDNA clone, a protein sequence of 279 amino acids was obtained (predicted molecular weight = 32.1 kDa) (Fig. 1). The isoelectric point of the predicted protein is 7.2 as determined by the software PROSIS. The cDNA clone was named human heart-specific four and a half LIM-only protein 2 (FHL2). It possesses an extra zinc finger that is a half LIM domain and four repeats of LIM domain. LIM domains are predicted to bind two molecules of Zn^{2+} to form two zinc-finger-like structures (Michelsen et al., 1993). Therefore, one molecule of FHL2 could bind up to nine Zn^{2+} and form nine zinc fingers. This FHL2 is unique because it possesses an odd number of zinc fingers as compared with other LIM-only proteins. When the LIM domains of FHL2 and other LIM proteins are aligned, some notable features can be observed (Fig. 2). The consensus sequence of the four LIM domains is $\mathbf{C}X_2\mathbf{C}X_3\mathbf{I}X_{11-15}\mathbf{WH}X_2\mathbf{CF}X\mathbf{C}X_2\mathbf{C}X_3\mathbf{(I/L)}X_4\mathbf{(F/Y)}X_8\mathbf{C}X_2\mathbf{C}$ (C, cysteine; H, histidine; I, isoleucine; W, tryptophan; F, phenylalanine; L, leucine; Y, tyrosine; X, any amino residues. The conserved amino-acid residues are represented by bold letters). The hydrophobic residues (I/L) and (F/Y) are present in many zinc fingers that have

PCR of FHL2 cDNA clone was performed by using a pair of primers flanking the open reading frame (ORF) of FHL2 (forward: 5′-TAGGGCGAATTCATGACTGAGCGCTTTGACTGCCA-3′; reverse: 5′-AGGGCGTCGACTCAGATGTCTTTCCCACAGTCGG-3′). Both primers have an end clamp (TAGGGC) which facilitated cleavage by restriction enzymes. An *Eco*RI site and an *Sal*I site are present in the forward and reverse primers, respectively. After digestion with *Eco*RI and *Sal*I, the PCR fragment was subcloned into the plasmid pGBT9.

Panel A:

Consensus sequence for LIM domain:

$CX_2CX_3IX_{11-15}WHX_2CFXCX_2CX_3(I/L)X_4(F/Y)X_8CX_2C$

Panel B:

```
                          *   *    *                      **  * *   *   *    *     *          *   *
HLOP (Zn finger)                                                FD CHHCNESLFGKKYILREESP      YCVVC FETLFANT
HLOP (LIM1)       CEECGKPIGCDCKDLSYKDRH           WHEAC FH CSQCRNSLVDKPFAAKEDQL      LCTDC YSNEYSSK
HLOP (LIM2)       CQECKKTIMPGTRKMEYKGSS           WHETC FI CHRCQQPIGTKSFIPKDNQN      FCVPC YEKQHAMQ
HLOP (LIM3)       CVQCKMPITTGGVTYREQP             WHKEC FV CTACRKQLSGQRFTARDDFA      YCLNC FCDLYAKK
HLOP (LIM4)       CAGCTNPISGLGGTKYISFEERQ         WHNDC FN CKKCSLSLVGRGFLTERDDI      LCPDC
DRAL (½LIM)                                             -- --------------------      ----- --------
DRAL (LIM1)       ---------------------           ----- -- --------------------      ----- --------
DRAL (LIM2)       ---------------------           ----- -- --------------------      ----- --------
DRAL (LIM3)       -----K-------------             ----- -- --------------------      ----- --------
DRAL (LIM4)       -----------------------         ----- -- --------------------      -----
SLIM3 (LIM2)                                            FI CHRCQQPIGTKSFIPKDNQN      FCVPC YEKQHAMQ
SLIM3 (LIM3)      CVQCKMPITTGGVTYREQP             WHKEC FV CTACRKQLSGQRFTARDDFA      YCLNC FCDLYAKK
SLIM3 (LIM4)      CAGCTNPISGLGGTKYISFEERQ         WHNDC FN CKKCSLSLVGRGFLTERDDI      LCPDC
hPAX (LIM1)       CGACKKPIAGQVVTAMGKT             WHPEH FV CTHCQEEIGSRNFFERDGQP      YCEKD YHNLFSPR
hPAX (LIM2)       CYYCNGPILDKVVTALDRT             WHPEH FF CAQCGAFFGPEGFHEKDGKA      YCRKD YFDMFAPK
hPAX (LIM3)       CGGCARAILENYISALNTL             WHPEC FV CRECFTPFVNGSFFEHDGQP      YCEVH YHERRGSL
hPAX (LIM4)       CSGCQKPITGRCITAMAKK             FHPEH FV CAFCLKQLNKGTFKEQNDKP      YCQNC
PINCH (LIM1)      CERCKGGFAPAEKIVNSNGEL           YHEQC FV CAQCFQQFPEGLFYEFEGRK      YCEHD FQMLFAPC
PINCH (LIM2)      CHQCGEFIIGRVIKAMNNS             WHPEC FR CDLCQEVLADIGFVKNAGRH      LCRPC HNREKARGLGKYI
PINCH (LIM3)      CQKCHAIIDEQPLIFKNDP             YHPDH FN CANCGKELTADARELKGEL       YCLPC HDKMGVPI
PINCH (LIM4)      CGACRRPIEGRVVNAMGKQ             WHVEH FV CAKCEKPFLGHRHYEKGLA       YCETH YNQLFGDV
PINCH (LIM5)      CFHCNRVIEGDVVSALNKA             WCVNC FA CSTCNTKLTLKNKFVEFDMKP     VCKKC
hZYX (LIM1)       CGRCHQPLARAQPAVRALGQL           FHIAC FT CHQCAQQLQGQQFYSLEGAP      YCEGC YTDTLEK
hZYX (LIM2)       CNTCGEPITDRMLRATGKA             YHPHC FT CVVCARPLEGTSFIVDQANRP     HCVPD YHKQYAPR
hZYX (LIM3)       CSVCSEPIMPEPGRDETVRVVALDKN      FHMKC YK CEDCGKPLSIEADDNGCFPLDGHV  LCRKC
LIN-11 (LIM1)     CAACAQPILDRYVFTVLGKC            WHQSC LR CCDCRAPMSMTCFSRDGLI       LCKTD FSRRYSQR
ISL-1  (LIM1)     CVGCGNQIHDQYILRVSPDLE           WHAAC LK CAECNQYLDESCTCFVRDGKT     YCKRD YIRLYGIK
MEC-3  (LIM1)     CNCCNEQIYDRFIYRMDNHS            YHENC VK CTICESPLAEKCFWKNGRI       YCSQH YYKDHSGIK
LIN-11 (LIM2)     CAGCFGKLEKEDLVRRARDKV           FHIRC FQ CSVCQRLLDTGDQLYIMEGNRF    VCQSD
ISL-1  (LIM2)     CAKCSIGFSKNDFVNRARSKV           YHIEC FR CVACSRLIPGDEFALREDGL      FCRAD
MEC-3  (LIM2)     CAGCKKGVSPTDMVYKLKAGLV          FHENC HC CSLCGRHLSPGEQILVDDTMMTV   SCMSH

                                        LIM domains                                          Spacer
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Fig. 2. (A) The consensus sequence of the LIM domains of FHL2. (B) Multiple sequence alignments of the LIM domains and spacers in FHL2, DRAL (Genini et al., 1996a), SLIM3 (Morgan and Madgwick, 1996a), human paxillin (Salgia et al., 1995), PINCH (Rearden, 1994), human zyxin (Macalma et al., 1996), *lin*-11, *Isl*-1 and *mec*-3 (Liebhaber et al., 1990). The positions of consensus amino-acid residues (*) are indicated. Amino-acid residues of DRAL that are identical with FHL2 are indicated by '—'.

two cysteines and two histidines, for example, ADR1 (Parraga et al., 1988) and *Xfin*-31 (Lee et al., 1989). Each LIM domain of FHL2 was separated by eight amino-acid residues. The role of the half LIM domain (extra zinc finger) remains to be clarified. The LIM protein that best fits the consensus sequence of FHL2 is human paxillin (Salgia et al., 1995), which is a focal adhesion protein and contains four tandem repeats of LIM domain.

When the DNA sequence of FHL2 was searched against the GenBank nucleotide databases, FHL2 was shown to be homologous with two DNA sequences. The first was a predicted partial cDNA sequence called skeletal muscle LIM-protein 3 (SLIM3) (Morgan and Madgwick, 1996a), which was assembled from five expressed sequence tags (accession numbers T39706, R57539, R57600, R57861 and T34559) in the non-redundant databases located at the National Centre for Biotechnology Information (NCBI). Neither start codon nor 5′ untranslated region could be found in the SLIM3 partial cDNA, which codes for only 153 amino-acid residues of the carboxyl terminal of FHL2 (Fig. 2). Another difference is that the SLIM3 sequence is incomplete at the 3′ untranslated region. The second homologous sequence was an unpublished DNA sequence encoding a LIM protein called DRAL (Genini et al., 1996a). The DRAL clone was isolated from a human neonatal skeletal muscle cDNA library. There was 96.7% identity between the cDNA sequences of FHL2 and DRAL (data not shown). Although the coding regions of FHL2 and DRAL are similar, the 5′ non-coding regions are very different from each other. When the amino-acid sequences of FHL2 and DRAL were aligned, a 99.6% identity between these two proteins was found (Fig. 2), with only a difference of one amino-acid residue. At position 167, methionine (M) in FHL2 was replaced by lysine (K) in DRAL. Previous studies have shown that DRAL cDNA is expressed in primary myoblasts but down-regulated in the embryonal-rhabdomyosarcoma (RMS) cell line RD (Genini et al., 1996b).

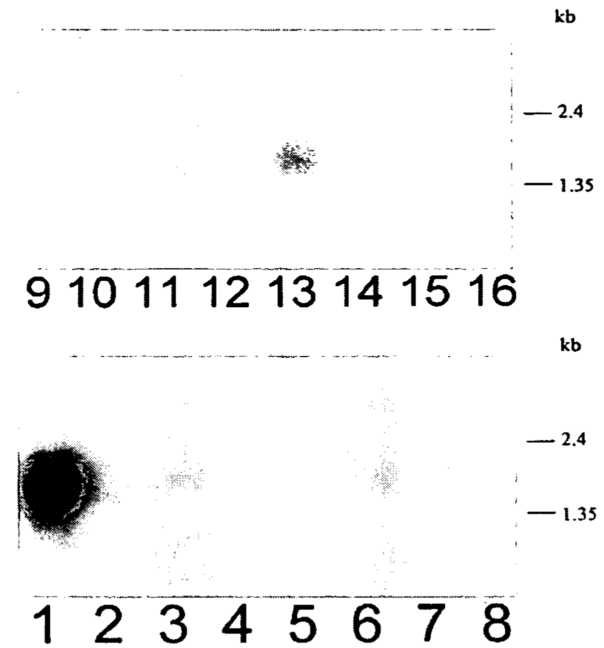

Fig. 3. Northern hybridization of FHL2 in human tissues. Key of the lanes: 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas; 9, spleen; 10, thymus; 11, prostate; 12, testis; 13, ovary; 14, small intestine; 15, colon; 16, peripheral blood leukocyte. **Methods**: Two Northern blots containing poly-A RNA from a variety of human tissues were obtained from Clontech Laboratories. Radioactively labeled random primed probe was made by using the purified PCR product of FHL2 as the template. Blots were prehybridized for 6 h and hybridized at 42°C for 18–20 h. Membranes were then washed in $1\times$SSC twice and washed again in $0.1\times$SSC with 0.1% SDS at 42°C to remove non-specific annealing. Autoradiography was performed at -70°C for 48–72 h.

Therefore, we believe that FHL2 may play a crucial role in the development and differentiation of human muscles.

2.3. Tissue distribution of FHL2

When the human FHL2 cDNA probe was used to hybridize with poly (A) RNA of various human tissues, the tissue distribution of the FHL2 mRNA was revealed (Fig. 3). It was shown that a very strong signal could be seen in the heart tissue. Only moderately low signals could be detected in placenta, skeletal muscle and ovary. Virtually no signal could be detected in brain, lung, liver, kidney, pancreas, spleen, thymus, prostate, testis, small intestine, colon or peripheral blood leukocyte. These results show that FHL2 is a LIM-only protein that is preferentially expressed in heart. When the cDNA sequence of FHL2 was searched against the database of expressed sequence tags (dbEST), 17 partial sequences matched with FHL2, 12 originated from fetal heart, two from adult heart, one from ovary, one from fetal spleen and one from senescent fibroblasts. This result supports our data showing that FHL2 is differentially expressed in heart. Previous results have shown that SLIM3 is expressed in human skeletal muscle (Morgan and Madgwick, 1996b). However, we have shown that heart has the highest level of FHL2 expression. Therefore, we suggest that FHL2 is a more appropriate name than SLIM3. Interestingly, MLP, which is a LIM-only protein with two LIM domains, is also highly expressed in the human heart. Its expression is enriched in striated muscle and occurs concomitantly with terminal muscle differentiation. Over-expression of MLP in C2C12 myoblasts promotes muscle differentriation, whereas antisense MLP prevent myogenesis (Arber et al., 1994). Similarly, MLP-deficient mice developed dilated cardiomyopathy with hypertrophy, heart failure and disruption of cardiomyocyte cytoarchitecture after birth (Arber et al., 1997). Since FHL2 has a high expression level in adult heart, we speculate that FHL2 may be particularly important for the maintenance of the heart phenotype. Besides, one of the LIM domains-LIM1 of MLP appears to play a role in nuclear localization, interacting with the muscle regulatory factors (MRFs) and enhancing the formation of MRF–DNA complexes, whereas another LIM domain-LIM2 primarily interacts with cytoplasmic proteins involved in maintaining the cellular architecture (Arber and Caroni, 1996; Kong et al., 1997). Thus, it might be possible that the LIM domains of FHL2 also function as specific adapter elements to promote the assembly and targeting of multiprotein complexes. Such speculation awaits further investigation in finding the protein partners of FHL2.

2.4. Chromosomal mapping of FHL2 gene

The FHL2 gene is located at chromosome 2q12–q13 (Fig. 4). No significant FISH signals were observed from other chromosomes. Also located in this region, according to the database of NCBI, are paired box homeotic gene 8 (Stapleton et al., 1993), engrailed homolog 1 (Kohler et al., 1993), interleukin 1 alpha (Lafage et al., 1989), interleukin 1 beta (Webb et al., 1986), interleukin 1 receptor alpha (Copeland et al., 1991), interleukin 1 receptor beta (McMahan et al., 1991), activin AB beta polypeptide (Barton et al., 1989), T-cell differentiation protein mal (Alonso et al., 1988), v-*ral* oncogene homolog B (Hsieh et al., 1990), 70-kDa tyrosine phosphoprotein (Ku et al., 1994), gibbon ape leukemia virus receptor 1 (Kaelbling et al., 1991), diazepam binding inhibitor (Mocchetti, 1990), nucleolin (Srivastava et al., 1990), protein C (Patracchini et al., 1989), lysosomal H^+ transporting ATPase (Ozcelik et al., 1991) and cytochrome c oxidase subunit Vb (Lomax et al., 1991). Moreover, familial juvenile nephronophthisis (Hildebrandt et al., 1995) and the pericentric inversion (Pallotta, 1991), inv(2)(p12q14), which results in a syndrome of iris coloboma, bilateral

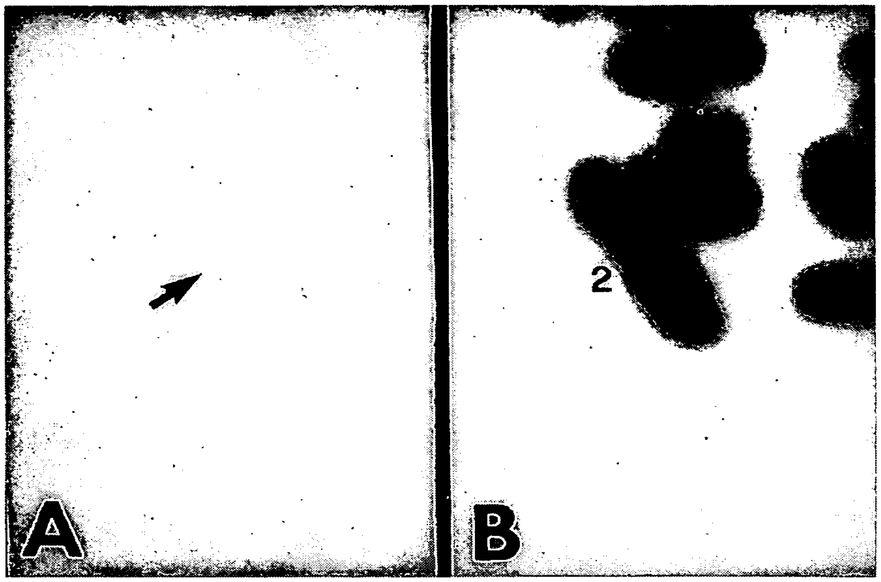

Fig. 4. FISH mapping. (A) FISH signals on chromosome. (B) Same mitotic figures stained with DAPI to identify chromosome 2. **Methods**: The chromosomal mapping of the FHL2 gene was performed by Dr H.H.Q. Heng of SeeDNA Biotech Inc. (Ontario, Canada). The pGBT9-FHL2 plasmid was biotinylated with dATP using the BRL BioNick labelling kit. The procedure for fluorescent in-situ hybridization (FISH) detection was performed essentially as described by Heng et al. (1992) and Heng and Tsui (1993).

oculotosis, hypertelorism, broad nasal bridge, and prominent epicanthic folds, have been mapped to this region. Recently, we have also mapped human FHL1 (alias: SLIM1), which has the same domain structure as FHL2, to chromosome X (manuscript in preparation). Therefore, although FHL2 and FHL1 belong to the same family of LIM proteins expressed in muscles (Morgan and Madgwick, 1996b), they are located at different regions of the human genome.

2.5. *Conclusions*

(1) A human fetal heart cDNA (1416 bp) encoding a novel LIM-only protein was isolated and characterized.
(2) The predicted ORF (encoding 279 aa) of this cDNA codes for the human heart-specific four and a half LIM-only protein 2 (FHL2). It possesses an extra zinc finger that is a half LIM domain and four repeats of LIM domain.
(3) The FHL2 is preferentially expressed in heart, only moderately expressed in placenta, skeletal muscle and ovary and there is virtually no expression in brain, lung, liver, kidney, pancreas, spleen, thymus, prostate, testis, small intestine, colon or peripheral blood leukocyte.
(4) The FHL2 was mapped to chromosome 2q12–q13 by FISH.

Acknowledgement

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EXHIBIT

2

Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

Search Nucleotide for Go Clear

Limits Preview/Index History Clipboard Details

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BLink, Domains, Links

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            annotation updated: Dec 15, 1998.
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  TITLE     Subtractive cloning and characterization of DRAL, a novel
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            the EMBL outstation - the European Bioinformatics Institute.
            The original entry is available from http://www.expasy.ch/sprot
            and http://www.ebi.ac.uk/sprot
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